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Article



Polymorphisms associated with the number of live-born piglets in sows infected with the PRRS virus in southern Sonora Mexico



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Abstract:

The porcine reproductive and respiratory syndrome (PRRS) is a viral disease that decreases the reproductive performance in breeding sows and leads to economic losses to the swine industry. The objective of the present study was to identify single nucleotide polymorphisms (SNP) associated to the number of live-born piglets in the first (LBP1) and second birth (LBP2) in breeding sows exposed to PRRS virus. The study included 100 pregnant females of the Landrace^(3/4)/ Yorkshire^(1/4) line, 75 of which were infected with the PRRS virus and 25 were free of PRRS. Individual blood samples (6-8 drops) were obtained and spotted onto FTA cards and subsequently processed for DNA extraction, which was genotyped using a 10,000 SNP chip for genomic profile. Resulting genotypes were analyzed using a multi-locus mixed model that detected three SNP associated to LBP1 and five SNP associated to LBP2 ($P<0.001$). These eight SNP were validated using an associative mixed effects model which included the terms genotype and age of dam as fixed effects, and sire as random effect. Allele substitution effects were estimated using the same model including the term genotype as covariate. The SNP rs81276080, rs81334603 and rs80947173 were associated to LBP1 ($P<0.001$), whereas the SNP rs81364943, rs80859829, rs80895640, rs80893794 and rs81245908 were associated to LBP2 ($P<0.001$). Only two SNP were in functional chromosomal regions and the remainder SNP were within an intergenic position. In conclusion, these results suggest the existence of gene variants associated with the reproductive performance of sows infected with the PRRS virus.

Key words: Alleles, Breeding sows, Genotype, Live-born piglets, PRRS, SNP.

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Introduction

The respiratory and reproductive syndrome (PRRS) is a worldwide disease that causes economic losses in the porcine industry estimated at approximately \$3.08 American dollars per pig at market⁽¹⁾.

The etiology agent of PRRS is an RNA virus of single chain belonging to Arterivirus gender, whose main characteristics are an elevated mutation rate that confers a high antigenic variability, and its capacity to induce persistent infections^(2,3). The initial report of PRRS

disease in Mexico described that infected sows showed a case of reproductive problem and mortality in the production line⁽⁴⁾. The first clinical, epidemiological and productive description of the disease, as well as the first isolation of the PRRS virus, were reported in the states of Mexico, Guanajuato, Veracruz and Puebla⁽⁵⁾.

The PRRS virus infection is characterized by poor feed conversion that leads to a low weight in pigs, as well as fertility alterations in breeding sows such as estrus return, fetal mortality, mummification, abortions induction and low viability of piglets at birth⁽¹⁾.

Vaccination is the most common method for PRRS control and currently it has achieved to prevent in some extent the PRRS infection. However, the efficiency of the vaccines is still far to be universal because of the virus has the ability to avoid the immune response of the host; moreover, there exist genetic differences among hosts in response to vaccine virus exposition^(6,7). The vaccination is able to show some efficiency against homologous PRRS strains, but its efficiency against heterologous strains is drastically reduced. Therefore, the vaccination against the PRRS virus at present only guarantee a reduction in the length of the viremia and the elimination of the virus cycle, as well as a decrease in the intensity of signs and the appearance of clinical symptoms⁽⁸⁾.

The existence of genetic variants associated to the interaction between the PRRS virus and the host, as well as the evidence of a natural variability in the tolerance and/or susceptibility to the PRRS in the commercial porcine lines, they are opened the door for using molecular technologies as a valuable tool to battle the PRRS disease⁽⁹⁾. In this regard, the marker assisted selection (MAS) can be used to study candidate genes in order to identify those animals that possess a superior genetic ability for the expression of economically important traits, which include resistance or tolerance to diseases⁽¹⁰⁾. First examples of the application of these technologies in pigs were the selection against the halothane gene, and the identification of a significant association between the estrogen receptor gene and the number of live-born piglets⁽¹¹⁾. However, the current development of more robust computer systems has allowed to perform the whole genome selection, which involves an extensive use of molecular markers that cover the entire genome, in such a way that hundreds of thousands of molecular variants can be simultaneously studied in order to explain the generic variation of a phenotypic trait⁽¹²⁾. This method allows to perform associative studies that include the simultaneous analysis of a great amount of markers through the use of low- (10k=10,000) or high-density devices (50k=50,000 to 60K=60,000 SNP).

Several studies have been developed in pigs with the objective to identify regions within the DNA related to economically important traits such as the resistance to the PRRS virus^(13,14,15). Initial reports suggest the existence of a genetic basis associated to the PRRS disease. In this regard, pigs from the breed Hampshire infected with PRRS showed pulmonary damages more serious than pigs from the breeds Duroc and Meishan⁽¹⁶⁾. Furthermore, pigs from the

synthetic line Large White-Landrace showed a lower rectal temperature and a reduction in the viremia after be infected with the PRRS virus, in comparison with pigs from the synthetic line Hampshire-Duroc⁽¹⁷⁾. Recently, it has been reported within the chromosome 4 a genomic region associated to the resistance to the PRRS virus, which evidenced the existence of a strong genetic component associated to such ability⁽³⁾.

Currently, there is scarce information that report genes and/or genetic variants related to the phenotypic differences observed in the reproductive efficiency of sows infected with the PRRS virus. Therefore, the genetic foundation analyses of the reproductive response of these sows could lead to the identification of genetic markers associated to an appropriate reproductive performance, which would be very useful for the implementation of more efficient selection programs that include sows with superior genetic ability to tolerate and/or resist the infection of the PRRS virus.

Based on the previous information, the objective of the present study was to identify single nucleotide polymorphisms associated to the number of live-born piglets in the first (LBP1) and second birth (LBP2) in breeding sows infected with the PRRS virus.

Material and methods

Location and experimental units

This study was performed in a full-cycle commercial porcine herd located in the Yaqui Valley, Sonora, Mexico (NL: 27°17', WL: 109°56'). The study included 100 breeding sows from the commercial line Landrace(3/4)/Yorkshire(1/4), 12-mo of age and proved to be free of PRRS disease.

Health and reproductive management

At 15 d after be admitted in the breeding area, 75 sows resulted as naturally infected with a wild strain of the PRRS virus (positive group; n= 75) because, even though the farm was PRRS-free at the beginning of the experiment, it was located within an PRRS endemic region affected by several Norte American strains (PRRSV NA). By the other hand, a negative control group was composed by 25 sows which were maintained free of PRRS infection (control; n= 25). This was confirmed by both serologic and molecular tests performed along

the experiment. The sows inside the breeding area started their reproductive management that consisted in providing two services after be observed in estrus, using boars with proved high-fertility. After be confirmed as pregnant, the sows were moved into the gestation area where they remained until the day before their programmed birth. At this time, the sows were moved again into the maternity area. Immediately after the birth, records for total number of piglets born, live-born piglets and dead-born piglets were collected and stored in the computer software PigWIN®. The same reproductive management and data collection described before was repeated for the second farrowing of each sow and its corresponding birth.

Laboratory analyses

Blood samples were individually collected through auricular vein puncture at d 7, 30, 120 and 240 after the sows came into the breeding area; the samples were used to the serum determination of specific antibody titles against the PRRS virus using the diagnostic tool “ELISA-IDEXX” (Enzyme Linked Immunoassay, Lab Inc.). The viral RNA was isolated from blood serum through an automatic extraction system of nucleic acids by magnetic separation (TACO System, Gene Reach Biotechnology Corporation). The RNA was purified and then analyzed by real-time PCR using a commercial kit (Tetracore Nextgen Real-Time QT-PCR) which recognizes an ORF-7 segment from the PRRS virus. Results were reported as the number of RNA copies from the PRRS virus per mL of sample (Cepheid Smart Cyclor V2.0d).

Genome wide association study

An additional blood sample (0.5 ml) was collected from each sow and spotted onto FTA blood cards for collection of nucleic acids. The cards were stored at 25°C and subsequently sent to Neogen Lab for DNA extraction, purification and quantification. The DNA was genotyped using a device of low-density genomic profile (LDPorcine BeadChip, Neogen®, Lincoln, NE) with capacity to analyze 10,000 single nucleotide polymorphisms (SNP). The software PLINK (V1.07)⁽¹⁸⁾ was used for quality control of genotyping results, which consisted in the elimination of SNP with genotyping call rate below 90 %, minor allele frequency lower than 5 % and Mendelian error rate higher than 0.1. After the quality control study, a total of 8,826 SNP resulted useful and informative for the genome-wide association study. To do this, a multi-locus mixed model was constructed in order to identify SNP associated with the reproductive traits LBP1 and LBP2, using the software Golden Helix

SVS 7 (Golden Helix Inc., Bozeman, Montana, USA). The “stepwise” procedure was performed to identify the significant SNP as fixed effect covariables. In addition, the model allowed to use a matrix for genomic relationships estimated from the available genotypes (SNP) for each animal. The SNP considered as associated to the evaluated phenotypes were those with $\alpha=0.001$, and all SNP resulted as significant ($P<0.001$) were retained for validation analyses.

Statistical analyses

Descriptive statistics for the variables total born piglets, live-born piglets and dead-born piglets were calculated through the procedure MEANS from the statistical software SAS version 9.4 (SAS Inst. Inc., Cary, NC). An analyses of variance was utilized to determine if the variables mentioned before differed between sows positive and negative to PRRS disease ($P<0.05$) using the procedure PROC GLM. Normality and variance equality tests were performed using the procedure UNIVARIATE⁽¹⁹⁾.

Validation of genetic markers associated to LBP1 and LBP2

The procedure ALLELE was used to calculate allelic and genotypic frequencies, and to perform Chi-squared (X^2) test to verify possible deviations from the Hardy-Weimberg equilibrium⁽²⁰⁾. The SNP that resulted associated ($P<0.001$) to the traits LBP1 and LBP2, and accomplished with the criteria of minor allele frequency higher than 10 % ($FAM>0.10$) and no-deviation from the Hardy-Weimberg equilibrium ($X^2>0.05$), were subjected to a validation analysis trough a genotype to phenotype association study, using the procedure MIXED for variables of continue distribution. Such analysis of individual validation for each SNP was performed trough a mixed effects model, which included fixed effects of polymorphism genotype and age of dam, the random effect of the sire (i.e., using Z statistics to test if $H_0:\delta w^2=0$) and the residual effect (mean=zero, variance= δe^2).

Comparisons among means from the SNP genotypes associated with the traits LBP1 and LBP2 were obtained using the option PDIF of the procedure LSMEANS, including the Bonferroni adjustment provided that genotype term resulted as significant source of variation ($P<0.05$) in the associative analysis. Substitution allelic effects were estimated using the mixed effects model previously described, which included for this analysis the term genotype as covariable⁽²¹⁾.

Results

Variables associated to PRRS

Descriptive statistics for reproductive traits analyzed in this study are showed in Table 1, as well as variables related to viability of piglets at birth and at weaning. Average values for the variables total piglets born, and live-born piglets at first and second births were significantly ($P<0.01$) lower for sows positive to PRRS compared to negative sows (control), whereas an opposite effect was observed for the variable dead-born piglets, which suggests such variables are associated to the infection of the PRRS virus in the present study.

Table 1: Average values \pm SE for reproductive and viability traits associated with first and second births in reproductive sows positive and negative to PRRS

Trait	Positive to PRRS		Negative to PRRS	
	N	Average \pm SE	N	Average \pm SE
First birth:				
Total born piglets	75	11.24 \pm 3.12 ^a	25	12.65 \pm 3.56 ^b
Live-born piglets	75	10.17 \pm 3.27 ^a	25	11.48 \pm 3.59 ^b
Dead-born piglets	75	1.16 \pm 1.01 ^a	25	0.91 \pm 1.02 ^b
Number of weaning piglets	75	10.03 \pm 3.02	25	10.96 \pm 3.04
Weaned piglets total weight (kg)	75	56.67 \pm 13.05	25	67.84 \pm 13.01
Weaned piglets average weight (kg)	75	5.65 \pm 0.86	25	6.19 \pm 0.85
Second birth:				
Total born piglets	75	10.67 \pm 3.02 ^a	25	11.92 \pm 3.10 ^b
Live-born piglets	75	9.85 \pm 3.25 ^a	25	10.75 \pm 3.59 ^b
Dead-born piglets	75	0.94 \pm 1.02 ^a	25	0.75 \pm 1.03 ^b
Number of weaning piglets	75	9.76 \pm 1.64	25	10.92 \pm 1.79
Weaned piglets total weight (kg)	75	48.89 \pm 14.62	25	59.76 \pm 14.51
Weaned piglets average weight (kg)	75	5.01 \pm 0.84	25	5.47 \pm 0.81

Genome-wide association study

A total of 8,856 SNP fulfilled the criteria of quality to be included in the associative genomic analyses that detected genomic regions associated to LBP1 (chromosomes 6 and 7; Figure 1)

and to LBP2 (chromosomes 2, 5, 7 and 8; Figure 2), which explain 3.6 and 4.1 % of the variation associated to the traits LBP1 and LBP2, respectively. The individual genomic analyses detected three SNP associated to the variable LBP1 ($P<0.001$) and five SNP associated to the variable LBP2 ($P<0.001$). Only two of the eight SNP mentioned before are located within functional gene regions (introns), the rs81276080 within the gen TTR (Transthyretin) and the rs80893794 within the gen CWH43 (Cell wall biogenesis 43 C-terminal homolog). The record of the eight SNP detected as associated to the traits LBP1 and LBP2, as well as the genes and biological processes related to such SNP, are showed in Table 2.

Figure 1: Manhattan plot showing the position of the SNP associated with the trait of LBP1. (significance threshold fixed to $P<0.001$)

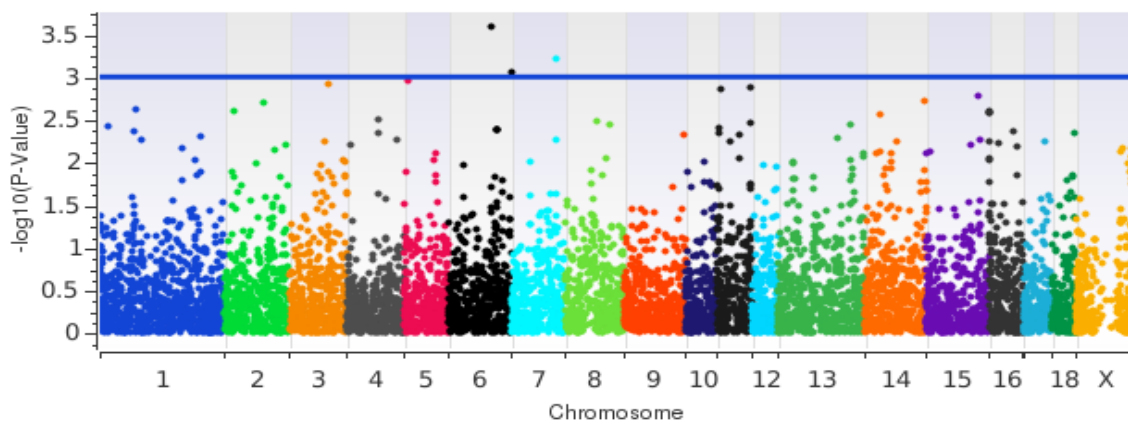


Figure 2: Manhattan plot showing the position of the SNP associated with the trait of LBP2. (significance threshold fixed to $P<0.001$)

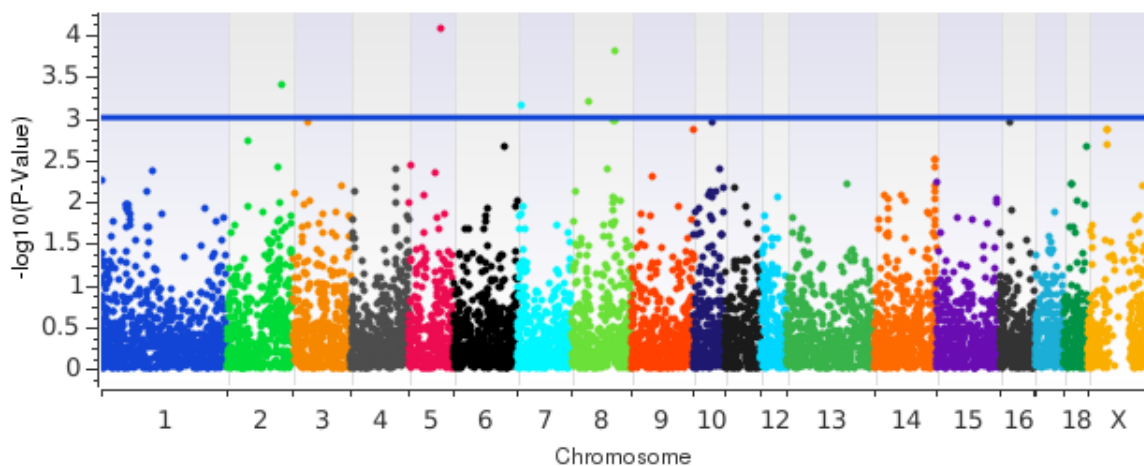


Table 2: Genes and biological processes related to the SNP associated with the traits of LBP1 and LBP2 in breeding sows positive and negative to PRRS

Trait	SNP	Variant	Associated gene	Associated biological process
LBP1	rs81276080	Intronic	TTR	Hormonal activation
	rs81334603	Intergenic	KCNQ4	Neuronal transmission
			CTPS1	Immune response
LBP2	rs80947173	Intergenic	FLRT2	Neuronal development
	rs81364943	Intergenic	ISOC1	Oogenesis
			ADAMTS19	Enzymatic activation
	rs80859829	Intergenic	IRAK4	Immune response
			ADAMTS20	Enzymatic activation
	rs80895640	Intergenic	CD83	Immune response
	rs80893794	Intronic	CWH43	Cellular activation
	rs81245908	Intergenic	FAT4	Neuronal transmission

LBP1= Live-born piglets at 1st birth; LBP2= Live-born piglets at 2nd birth.

Validation of genetic markers

The eight SNP identified due to its association with the reproductive traits of LBP1 and LBP2 fulfilled the criteria for no-deviation of the Hardy-Weinberg equilibrium ($X^2=1.0$, $P>0.28$) and minor allele frequency higher than 10 % ($MAF>0.10$; Table 3); therefore, these SNP were considered as candidate genes in this study. Three of these SNP were validated as predictors of LBP1 ($P<0.001$) and the other five SNP were validated as predictors of LBP2 ($P<0.001$). Table 4 shows the least square means for the genotypes of each SNP associated to the variables of LBP1 and LBP2. The most favorable SNP were rs81334603 and rs81364943 because they showed the higher values for LBP1 ($GG= 13.47 \pm 1.11$) and LBP2 ($TT= 16.30 \pm 3.01$), respectively, whereas the less favorable SNP were rs81276080 and rs80859829 because they showed the lower values for LBP1 ($GG= 7.59 \pm 0.61$) and LBP2 ($CC= 5.94 \pm 0.78$), respectively. However, the eight SNP validated in this study showed a favorable genotype associated with the analyzed reproductive traits.

Table 3: Allelic and genotypic frequencies of the SNP associated with LBP1 and LBP2 in breeding sows positive and negative to PRRS

Trait	SNP	Position	Allelic Frequency		Genotypic Frequency		
			G	T	GG	GT	TT
LBP1	rs81276080	SSA 6	0.1878	0.8122	0.0352	0.3052	0.6596
			A	G	AA	AG	GG
	rs81334603	SSA 6	0.3867	0.6133	0.1467	0.4800	0.3733
			A	C	AA	AC	CC
	rs80947173	SSA 7	0.7431	0.2569	0.5556	0.3750	0.0694
			C	T	CC	CT	TT
LBP2	rs81364943	SSA 2	0.8266	0.1734	0.6800	0.2933	0.026
	rs80859829	SSA 5	0.3133	0.6867	0.0533	0.5200	0.4267
	rs80895640	SSA 7	0.3379	0.6621	0.1622	0.3514	0.4865
	rs80893794	SSA 8	0.3099	0.6901	0.1268	0.3662	0.5070
			A	G	AA	AG	GG
	rs81245908	SSA 8	0.3000	0.7000	0.0667	0.4667	0.4667

LBP1= Live-born piglets at 1st birth; LBP2² = Live-born piglets at 2nd birth.**Table 4:** Least square means \pm SE for genotypes of the SNP associated with the traits LBP1 and LBP2 in breeding sows positive and negative to PRRS

Trait	SNP	N	Means by genotype \pm SE			Prob
			TT	TG	GG	
LBP1	rs81276080	100	12.33 \pm 1.16 ^a	8.04 \pm 1.08 ^b	7.59 \pm 0.61 ^b	.0001
			GG	AG	AA	
	rs81334603	100	13.47 \pm 1.11 ^a	10.51 \pm 0.75 ^b	8.56 \pm 0.77 ^b	.0001
			AA	AC	CC	
	rs80947173	100	12.59 \pm 1.48 ^a	9.81 \pm 0.94 ^b	8.31 \pm 0.68 ^b	.0001
			TT	TC	CC	
LBP2	rs81364943	100	16.30 \pm 3.01 ^a	12.94 \pm 0.95 ^a	9.23 \pm 0.62 ^b	.0001
			TT	TC	CC	
	rs80859829	100	12.21 \pm 3.04 ^a	8.75 \pm 0.69 ^b	5.94 \pm 0.78 ^b	.0001
			TT	TC	CC	
	rs80895640	100	11.88 \pm 1.06 ^a	9.61 \pm 0.91 ^b	8.11 \pm 0.85 ^b	.0001
			CC	TC	TT	
	rs80893794	100	13.42 \pm 1.07 ^a	10.08 \pm 0.74 ^b	7.95 \pm 0.84 ^c	.0001
			GG	GA	AA	
	rs81245908	100	12.25 \pm 2.08 ^a	8.57 \pm 0.74 ^b	7.50 \pm 0.75 ^b	.0001

LBP1= Live-born piglets at 1st birth; LBP2= Live-born piglets at 2nd birth.

The substitution allelic effects for each SNP are showed in Table 5. The favorable alleles of the SNP rs81276080, rs81334603 and rs80947173 associated to LBP1 were T, G and A, because they increase 3.28 ± 0.74 , 3.52 ± 0.62 and 2.35 ± 0.68 the number of LBP1, respectively ($P < 0.001$). By the other hand, for the SNP rs81364943, rs80859829, rs80895640, rs80893794 and rs81245908 associated with LBP2, the favorable alleles were T, T, T, C and G, because they increase 3.66 ± 0.85 , 3.38 ± 0.82 , 1.92 ± 0.58 , 2.64 ± 0.61 and 3.18 ± 0.77 the number of LBP2, respectively ($P < 0.001$). The results described before indicate a favorable contribution of the eight validated SNP for the reproductive traits evaluated in the sows included in this study.

Table 5: Allelic substitution effects for SNP associated with the traits LBP1 and LBP2 in breeding sows positive and negative to PRRS

Trait	SNP	Favorable allele	Allelic Substitution Effect		
			Prob	Estimated value	SE
LBP1	rs81276080	T	0.0005	3.28	1.1476
	rs81334603	G	0.0002	3.52	0.6227
	rs80947173	A	0.0013	2.35	0.6870
LBP2	rs81364943	T	0.0001	3.66	0.8525
	rs80859829	T	0.0002	3.38	0.8201
	rs80895640	T	0.0022	1.92	0.5893
	rs80893794	C	0.0001	2.64	0.6162
	rs81245908	G	0.0002	3.18	0.7732

LBP1= Live-born piglets at 1st birth; LBP2= Live-born piglets at 2nd birth.

Discussion

The negative effect of the PRRS virus infection on the number of live-born piglets observed in the present study has been previously reported in sows from different parity^(22,23). In a similar study conducted with PRRS infected sows which were compared to healthy sows, it was observed an increase in the average values of mummified and non-born piglets of 0.04 to 1.12 and 0.62 to 1.02, respectively, as well as a reduction in the number of live-born piglets of 10.3 to 9.8⁽²⁴⁾.

Furthermore, the existence of genetic variability associated to the reproductive performance in sows infected with the PRRS virus has been also described in previous research reports. In this regard, Rashidi *et al*⁽¹⁵⁾ reported a variation of 3.83 ± 0.31 in the number of live-born piglets infected with the PRRS virus, compared to a variation of 1.96 ± 0.06 observed in

healthy sows. Such variability, mainly in sows infected with PRRS, suggests the existence of a genetic basis associated to the reproductive response in the face of the disease. Therefore, it has been pointed out as an important strategy to reduce the negative impact of the PRRS in breeding sows, the identification of molecular markers that allow a better understanding about the genetic control of the response to the virus, which would be eventually incorporated in marker assisted selection (MAS) programs⁽²⁵⁾.

In the present study, the genome-wide analyses identified genomic regions and a total of eight SNP associated to the variables LBP1 and LBP2 ($P < 0.001$) in breeding sows infected and non-infected with the PRRS virus. Such SNP showed a minor allele frequency greater than 10 %, which in general terms is considered as a requirement to avoid false results in genotype to phenotype association studies⁽²⁶⁾.

In the individual statistical analysis, the eight SNP previously identified were validated as predictors for the variables LBP1 and LBP2, from which only two of them are located within functional gene regions. On the one hand, the SNP rs81276080 (associated with LBP1) is located within the 5' region from the TTR gene (Transthyretin), which codifies the synthesis of a transport protein of thyroid hormones in plasma and cerebrospinal fluid. The TTR gene has been proposed as a potential candidate gene associated with the physiological response in pigs exposed to heat stress, which seriously restrict their reproductive performance⁽²⁷⁾, because it reduces oocyte quality and embryo viability, and enhances the PRRS negative effects on fertility of infected sows. On the other hand, the SNP rs80893794 (associated with LBP2) is located within an intron region from the gene CWH43 (Cell wall biogenesis 43 C-terminal homolog). This gene is involved in lipids remodeling of the cell wall from yeasts⁽²⁸⁾, and it has been reported that this gene is homologous to the gene PGAP2 (Post-GPI attachment to proteins), which is involved in both di-acetylation and re-acetylation cycles of the proteins that synthesizes Phosphatidyl Inositol (PI) in mammal cells⁽²⁹⁾. The PI is a family of lipids that participates in the second messenger mechanism in the cell membrane. This mechanism is used by several hormones such as PGF2 α which plays an important role in ovary function and uterine activity; then, it influences directly the variables LBP1 and LBP2.

The remaining six SNP are located within intergenic regions (positional). In this regard, is important to point out that, when exploring the entire genome, it is complicated to detect a causal variant or a variant directly responsible of phenotypic changes within populations; however, because of the property of linkage disequilibrium in the genome, it is possible to identify indirect associations among the SNP and specific phenotypes. This information supports the importance for considering genes whose chromosomal location is close to resulting significant SNP that possess an intergenic position (at least in a range of 100 thousand of base pairs; 100 kbp)⁽³⁰⁾. One of these SNP is the rs81334603 associated to LBP1, which is located to a distance of 40.26 kbp from the gene KCNQ4 (Potassium voltage-gated channel subfamily Q member 4). This gene is under-expressed in tracheobronchial lymph

nodes from pigs infected with the North American strain VR-2332 of the PRRS virus⁽³¹⁾. Moreover, approximately to 72.77 kbp from the SNP rs81334603 is located the gene CTPS1 (CTP Synthase 1), which codifies the production of the enzyme CTP synthase; the function of this enzyme is the biosynthesis of pyrimidine nucleotides (UTP and CTP), as well as the synthesis of ciclopentenilcitosine, a wide-spectrum antiviral agent⁽³²⁾.

In relation to the SNP rs80947173, also associated to LBP1, the closest gene to this SNP is the FLRT2 (Fibronectin leucine rich transmembrane protein 2) gene which is located at 889.05 kbp of distance. Even though it is true that this gene is located considerably far away from the SNP rs80947173, useful levels of linkage disequilibrium (>0.3) appear to extend in pigs to a higher distance than Holstein cows, which implies that low-density SNP panels could provide reliably results in genome-wide association studies⁽³³⁾. In addition, it has been reported that the gene FLRT2 is associated to the number of live-born piglets in populations of Large White and Landrace pigs⁽³⁴⁾.

In the case of the SNP associated to LBP2, one of them is the rs81364943, which is located to a distance of 88.74 kbp from the gene ISOC1 (isochorismatase domain containing 1) and 178.67 kbp from the gene ADAMTS19 (ADAM metalloproteinase with thrombospondin type 1 motif 19). The gene ISOC1 has been linked to a processes of catalytic activity in porcine oocytes according to a genetic co-expression study⁽³⁵⁾, whereas polymorphisms from the ADAMTS19 gene in women have been associated to the presence of the polycystic ovarian syndrome⁽³⁶⁾.

Another SNP associated with LBP2 was the rs80859829 which is located to 31.75 and 177.73 kbp from the genes ADAMTS20 (ADAM metalloproteinase with thrombospondin type 1 motif 20) and IRAK4 (Interleukin 1 receptor associated kinase 4), respectively. The possible explanation for the association of this polymorphism with the reproductive performance at birth in PRRS infected sows is because the gene ADAMTS20 is over-expressed in organs such as brain and gonads⁽³⁷⁾, which are affected after the infection of the PRRS virus⁽³⁸⁾. Likewise, the kinase associated with the interleukine-1 receptor (protein product from the gene IRAK4), it has been involved in the mechanisms of PRRS virus replication because its production is reduced by the action of a well-known micro RNA (miRNA-373) with proviral effects⁽³⁹⁾.

The SNP rs80895640 also associated with LBP2 is located to 34.3 kbp from the gene CD83 (CD83 molecule); this gene has been previously linked with the number of total born piglets and live-born piglets in hybrids pigs Iberic X Meishan^(40,41). Interestingly, in the immunological context, the CD83 molecule has been recently indicated as a key piece of the scape mechanisms of the PRRS virus against the immune system, because this virus is able to regulate positively the expression in soluble form of the molecule CD83 (sCD83), which was associated to the immunosuppression of T-cell proliferation in the host⁽⁴²⁾. Similarly,

from 54.05 kbp of the SNP rs80895640 is located the gene RNF182, which is involved in neuronal apoptosis processes⁽⁴³⁾ that commonly occur after the infection of highly-infectious strains of the PRRS virus⁽⁴⁴⁾.

Finally, the SNP rs81245908 (associated with LBP2) is located to an approximate distance of 53.52 kbp from the gene FAT4 (FAT atypical cadherin 4). The association of this polymorphism could be explained from a study which provides evidence that FAT4 gene expression in humans has been detected in fetal and infant brain tissues⁽⁴⁵⁾, because it has been also proved that PRRS maternal infection is able to affect neuronal development in piglets, reducing the number of neurons from the hippocampus and increasing the number of glia⁽⁴⁶⁾.

Conclusions and implications

The detection of genomic regions that explain 3.6 and 4.2 % of the variance associated to the traits live-born piglets at first and second births, as well as the validation of eight SNP located within such regions, suggest the existence of a genetic basis that underlies the reproductive response in breeding sows infected with the PRRS virus. Therefore, this study proposes to consider these eight SNP associated with the variables LBP1 and LBP2, two functional and six positional, as genetic markers for selection programs focused to improve the reproductive efficiency in sows infected with the PRRS virus. It is suggested to conduct additional studies to evaluate the functionality of the detected SNP; in addition, it is important to consider the validation of the genomic regions and genes reported in this study in other breed populations of sows also infected with the PRRS virus.

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